

THE HISTOLOGY OF THE RETRACTOR MUSCLES OF THYONE BRIAREUS LESUEUR

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The histology of invertebrate muscle has received relatively little investigation and there exist at the present time marked gaps in our knowledge of this subject. A more critical study of invertebrate muscle might possibly yield clues of interesting phylogenetic significance. Recent research in the field of holothurian muscle physiology has also made desirable a more complete histological study of these muscles.

The only extensive paper on the histology of holothurian muscle (Hall, 1927, on *Cucumaria*) completely misinterprets the essential structure and has been responsible for a number of erroneous conclusions by workers in muscle physiology.

This paper concerns itself with the more salient features of the histology of *Thyone* retractor muscle. The interpretations are opposed to the conclusions of Hall on the strikingly similar muscles of *Cucumaria*.

MATERIALS AND METHODS

The retractor muscles of *Thyone* occur in five main bands which run from the lantern to points of attachment on the five longitudinal muscles of the body. The individual bands may be single or broken up into a number of divisions. They are extremely extensible and in adult specimens may vary in length from about 5 mm. in the contracted condition to 5 or 6 cm. in normal extension. Except for the two regions of attachment the muscles are free of the tissue and are easily removed.

The muscles were fixed in varying degrees of contraction in Bouin's or Helly's fixing fluids. In order to obtain normal extension it was necessary to immerse them for some time prior to fixation in 0.3 molar magnesium chloride solution. The muscles were dehydrated in alcohol, cleared in xylene, and imbedded in paraffin. Sections were cut at 5 and 10 micra.

Heidenhain's iron hæmatoxylin proved to be the most useful stain for the muscle fibers. Mallory's aniline blue and Dominici's

stain were most favorable for the details of the connective tissue network.

Attempts were made to macerate the muscles in Bouin's fluid for periods up to three weeks and in varying strengths of nitric acid. These techniques, however, did not prove to be very satisfactory.

OBSERVATIONS

Microscopic examination of the fresh muscle reveals extremely long fibers grouped into indistinct bundles. In cross-sections of fixed and stained muscles this bundle arrangement is clearly discernible (Figs. 1 and 4). The inner portion of the muscle consists of hemicylindrical bundles containing from two to fifteen fibers which tend to be arranged about the periphery of each bundle. The outer portion of the muscle exhibits no definite bundles. Longitudinal sections reveal that the bundles do not maintain the same complement of fibers throughout their length. Fibers pass from one to the other of adjacent bundles and in the periphery of the muscle there is no definite association of fibers.

The spaces between the bundles and the fibers of the bundles are occupied by a delicate reticulum of connective tissue which is markedly condensed about each bundle (Figs. 1 and 4). The connective tissue appears to have the nature of a lattice-like framework with the thickest fibers running perpendicular to the muscle fibers. In sections of contracted muscle the elongated connective tissue nuclei are also seen to lie with their long axes in this direction. The connective tissue fibers are especially coarse and dense in the periphery of the muscle.

Connective tissue and tissue space constitute a relatively large proportion of the muscle in contrast to mammalian smooth muscle and some invertebrate muscle, e.g. retractor muscle of *Phascolosoma*. Steinbach (1937), in his studies on potassium and chloride in *Thyone* muscle, has estimated the volumes occupied by the muscle elements and tissue space. According to Steinbach, chloride diffuses freely into 43 per cent of the muscle—a figure which by his interpretation corresponds rather closely to the extrafibrillar space (tissue space and sarcoplasm). However, since Steinbach tentatively accepted the histological conclusions of Hall, his results may be reinterpreted. The structures which Steinbach, following Hall, interpreted as fibrils are actually fibers. Those structures interpreted as fibers are bundles of fibers. Thus it is found that chloride space corresponds closely to tissue space (47 per cent of *Thyone* muscle) as has commonly been believed (see Fenn, 1936), and does not include the sarcoplasm of the muscle fibers.

Fischer (1937) found that *Thyone* retractor muscle exhibited a birefringence of 60 per cent of that of *Phascolosoma* retractor muscle. (Histologically, *Phascolosoma* retractor muscle exhibits densely packed elements.) Since birefringence may be presumed to be associated with contractile elements, Fischer's figure suggests that these elements are fewer in number than in *Phascolosoma* muscle, and it may be further interpreted as lending support to the figure given above for tissue and chloride space in *Thyone* muscle.

As far as it has been possible to determine, the fibers are uni-nucleate. The nuclei occupy lateral positions on the fibers (Figs. 1 and 3), only rarely are they found within them (Fig. 4). In the contracted fiber the nuclei are rounded and are usually found to occupy a typical lateral position on the fiber. With the proper staining technique a layer of faintly staining cytoplasm may be seen to surround the nuclei. Often a thin strand of this cytoplasm forms the only connection between the nucleus and the muscle fiber. In the extended muscle the nuclei are elongated in the axes of the fibers and lie closely applied to them.

The appearance of the fibers in cross-section is somewhat variable. In the contracted condition they tend to be circular or semicircular (Fig. 1). Occasional fibers are polygonal. The stretched fibers may be circular or oval in transverse section, but are more often flattened or angular (Fig. 4). The fiber diameter varies from 5–10 μ in contraction to 2–4 μ in extension.

Teased preparations of muscle reveal the spindle-shaped character of the fibers. The tips of the fibers are long and tapering in extension (Fig. 5), bluntly rounded in contraction.

It was extremely difficult to determine the lengths of the muscle fibers since it was almost impossible to isolate these elements. The closely investing connective tissue binds the fibers securely together and all attempts at maceration and teasing were largely unsuccessful. Careful study of the best of these preparations, however, revealed that the fibers do not extend the whole length of the muscle. At

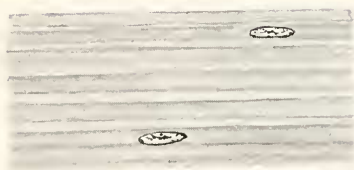
Explanation of Figures

All drawings were made with the aid of a camera lucida at a magnification of $\times 745$.

- FIG. 1. Cross-sections of two bundles of fibers from a contracted muscle.
- FIG. 2. Portion of a longitudinal section of an extended muscle.
- FIG. 3. Portion of a longitudinal section of a contracted muscle.
- FIG. 4. Cross-sections of two bundles of fibers from an extended muscle.
- FIG. 5. Fragment of a teased muscle.



1



2



4



3



5

various points along a teased muscle natural unbroken tapering fiber terminations could be found (Fig. 5).

DISCUSSION

The contention of Hall (1927) that *Cucumaria* muscle consists of large fibers containing a few giant fibrils forms an untenable theory. From Hall's figures it is apparent that the histological picture of *Cucumaria* muscle is almost identical with that of *Thyone*. In *Thyone* muscle, however, the nucleated condition of the so-called fibrils is clearly evident. It must be concluded, therefore, that these are muscle fibers and not fibrils. It is true that the nuclei often have a tenuous attachment to the fibers, but this attachment is nevertheless definite, and, as previously stated, nuclei are occasionally found within the fibers. The free nuclei within the bundles, which Hall interpreted as muscle nuclei, are clearly constituents of the connective tissue. Further, the failure of the peripheral elements to be arranged into bundles (muscle fibers of Hall) does not support Hall's conclusion that these are fibrils.

The conception that the muscle fibers are long spindle-shaped elements is supported by the studies of Gerould (1896) on *Caudina* and Jordan (1914) on *Holothuria* and *Stichopus*. According to Gerould, muscle fiber nuclei in *Caudina* occupy a lateral position similar to those in *Thyone*.

du Buy (1936), studying the sensitivity of *Thyone* muscle to certain drugs, found that these muscles exhibited a physiological condition intermediate between vertebrate smooth and vertebrate striated muscle. Microscopically they resemble vertebrate smooth muscle. No histological similarity with vertebrate striated muscle can be detected with the possible exception of their extreme length. The fibers are homogeneous, devoid of striations or fibrillar structure.

SUMMARY

The retractor muscles of *Thyone* consist of long spindle-shaped smooth muscle fibers. The fibers appear to be uninucleate. The nuclei usually occupy a lateral position and are attached to the fiber by a delicate layer of cytoplasm. Occasional nuclei are found completely within the fibers.

In the center of the muscle the fibers occur in bundles of two to fifteen. No such organization is exhibited in the periphery of the muscle.

The fibers do not remain associated with a single bundle throughout their length but pass freely from one bundle to the other.

Measurements of fiber length were not obtained, but it is evident from study of teased preparations that they do not extend the full length of the muscle. Fiber diameter varies from 5-10 micra in contraction to 2-4 micra in extension.

The connective tissue forms a lattice-like network which is concentrated about each bundle and in the periphery of the muscle. The heaviest fibers of the reticulum tend to run perpendicular to the axes of the muscle fibers.

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